J|A|C|S
COMMUNICATIONS

Published on Web 11/10/2004

Shape-Tunable Polymer Nodules Grown from Liposomes via Ring-Opening Metathesis Polymerization

Nathalie Jarroux,[†,‡] Patrick Keller,[†,⊥] Anne-Françoise Mingotaud,*[,§] Christophe Mingotaud,[§] and Cécile Sykes*[,†]

Laboratoire de Physicochimie "Curie", Institut Curie, UMR 168, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France, Laboratoire "Matériaux Polymères aux Interfaces", UMR 7581, Université d'Evry Val d'Essonne, UFR Sciences Fondamentales et Appliquées, Bd. F. Mitterrand, 91025 Evry Cedex, France, Laboratoire des Interactions Moléculaires et Réactivités Chimique et Photochimique, UMR 5623, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France, and Ferroelectric Liquid Crystals Material Research Center, Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309

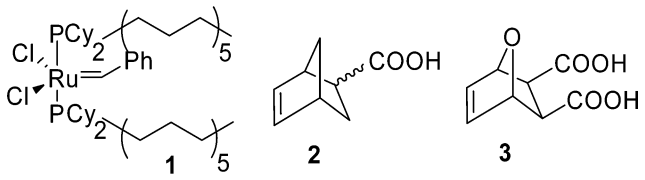
Received July 27, 2004; E-mail: cecile.sykes@curie.fr

Polymerization in lyotropic phases is a quickly expanding research area at the boundaries between various scientific domains (colloid, polymer, material, and biomedical science). In particular, the covalent assembly of the surfactants constituting the bilayers of liposomes has been developed in the past two decades[1−4] with the motivation of creating vectors with potential applications in drug delivery. Indeed, such a process confers strengthened mechanical properties to an initially soft and fluid surface. The reaction is based on the use of unsaturated phospholipids forming the liposome bilayers, and the polymerization reaction is usually of radical type and triggered photochemically. Another approach consists of polymerizing lipophilic monomers (styrene mainly) dissolved within the bilayers.[5] In that case, the reaction results in a polymer latex bead confined within the bilayer, which confers to the system the so-called "parachute architecture".

The strategy we develop here is different and reaches distinct goals. We insert a catalyst at the liposome membrane so that polymerization occurs on the external part of the liposome. As a result, the polymerizing chain is pushed away from the surface. The ultimate motivation for designing such a system is to biomimic the motility of living organisms or objects that move inside cells by polymerizing actin at their surface.[6,7] The design of an abiotic experimental system able to produce a movement generated by polymerization would be of great interest to physicists, chemists, and biologists, as it would provide a new simplified, versatile, and reproducible system.

In this work, we investigated the ring-opening metathesis polymerization (ROMP) of norbornene-type monomers dissolved in the outer aqueous phase of liposomes in the bilayers of which a specially designed hydrophobic derivative of the Grubbs' initiator has been incorporated.[8] We show that polymer nodules (up to 10 μm diameter) could be grown with a controllable shape at the surface of liposomes, making of this system one of the most productive surface-initiated polymerization systems described so far. The initiator **1** (Scheme 1) was designed in order to have a strong affinity for the hydrophobic part of vesicles and to maintain the catalytic center along the bilayer during the polymerization that occurred continuously at the surface of the liposome. It was obtained using the method previously described[9] (see Supporting Information). Liposomes were prepared by rehydration in a pH 8 buffer of a thin film obtained by drying a solution of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and initiator **1** (in molar ratio close to 20) in $CHCl_3$. The observed size of the liposomes was in the 1−4 μm range.

Scheme 1



The polymerization reaction occurred in a glovebox in the presence of an adequate amount of monomer **2** (5-norbornene-2 carboxylic acid) introduced via a syringe to a liposome solution contained in a 1-mm-thick UV cell (quartz or glass). Monomer **2** was soluble at pH 8 in our solutions. The volume of monomer was varied in order to modify the monomer/initiator ratio from 350 to 3500. The cell was sealed with a silicone stopper, mechanically shaken, and removed from the glovebox to allow observation under the microscope.

For a monomer/initiator **2/1** ratio of 3500, polymer formation was observed after a couple of hours at the surface of the liposomes. Decreasing the monomer/initiator ratio to 350 was sufficient to speed up polymerization for enabling shorter total reaction time (typically 1 h). Calcium ions were substituted to sodium ions in the aqueous phase in order to test whether physical cross-linking of the polycarboxylated polymer chains via divalent cations could induce changes in the shape, size, or compactness of the polymer nodules. Strikingly, substituting sodium in place of calcium (20 mM calcium in Tris buffer adjusted to pH 8) accelerated the reaction so that it occurred in less than 1 min. This marked effect on the kinetics of the ROMP might be due to a kind of template effect around the calcium ion. However, in all experiments described here, the addition of calcium did not result in any shape change. An example of such an experiment is given in Figure 1, where a large polymer mass is formed from several liposomes that are incorporated in the volume. Defocusing the microscope led to the observation that the polymer mass partially wets the bottom surface of the chamber, due to sedimentation.

To avoid this effect, we increased the density of the buffer solution by adding sucrose (13% w/w, 330 mM) and observed polymer nodules, the spherical shape of which was checked by defocusing the microscope. All other experiments using monomer **2** were therefore carried out in tetraborate buffer containing calcium ions at 0.1 mM, sodium ions at 19.9 mM, sucrose at 330 mM, and a ratio monomer/initiator of 3500 as optimized conditions for

[†] Institut Curie.
[‡] Université d'Evry Val d'Essonne.
[§] Université Paul Sabatier.
[⊥] University of Colorado.

10.1021/ja045482j CCC: $27.50

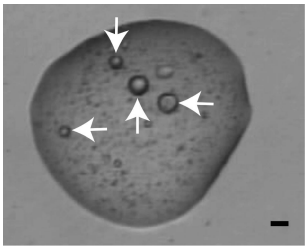


Figure 1. Optical microscopy image of a polymer (giant drop) obtained from liposomes (white arrows) after 11 min of polymerization. Bar, 20 μm.

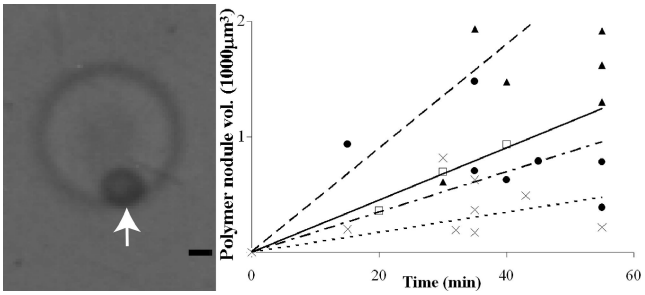


Figure 2. (Left) Optical microscopy image of a polymer (giant drop) obtained from monomer **2** on a liposome (white arrow) after 35 min of polymerization. Bar, 2 μm. (Right) Volume of the polymer nodule as a function of time: (□) one single liposome; different liposomes (1.25−1.8 μm (×), 1.8−2.4 μm (●), and 2.4−3.5 μm (▲)).

observing polymer growth from the liposomes in 3D conditions. Polymer nodules (one nodule by liposome) were thus obtained for 70% of the liposomes. As an example, a 2-μm-diameter liposome can lead to the growth of a polymer nodule of about 10 μm diameter (Figure 2, left). In these experiments also, the presence of calcium did not change the shape of the polymer nodule, which remained spherical. This could be explained by the hydrophobicity of the polymer and the effect of interfacial tension between the polymer and the external liquid.

To follow the growth of the polymer nodule as a function of time, we carried out a statistical kinetic study, since in most cases the presence of residual flow inside the cell chamber prevented us from following only a single liposome. From six experiments, several images were taken at different times and contained a number of liposomes from 1 to 6. The volume of the spherical nodules, measured by image analysis, is plotted in Figure 2 as a function of time for different sizes of liposomes.

We conclude from the plot in Figure 2 that the larger the liposome diameter, the larger the volume of the polymer nodule. This seems reasonable if initiator **1** is statistically distributed in the liposomes. We were able to follow some single objects, one example being displayed in Figure 2 (right) for a liposome of 2.1 μm diameter. The corresponding rate of nodule growth is about 22 μm^3/min, lower than that for liposomes in the 2.5−3.5 μm range, and higher than that for liposomes in the 1.25−1.75 μm range.

The polymer thus obtained was characterized by size exclusion chromatography using 2 mL of liposome solution in the same conditions of polymerization. To cut out the polymer chains from the initiator molecules, 50 μL of ethyl vinyl ether was added after 1 h of polymerization, and the polymer was purified by five successive precipitation and sedimentation operations using dichloromethane to remove phospholipids. The relative molecular weight, determined versus poly(ethyleneoxide) standards, was around 800 000 g mol^{-1}.

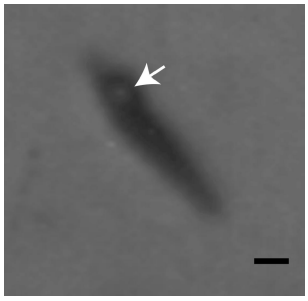


Figure 3. Optical microscopy image of a polymer (elongated dark gray form) obtained from monomer **3** on a liposome (white arrow) after 50 min of polymerization. Bar, 2 μm.

In all experiments performed heretofore, the polymer nodules developed as spheres, probably due to their relative hydrophobicity. To test this assumption, we synthesized the superhydrophilic monomer oxonorbornene dicarboxylic acid[10] (**3** in Scheme 1) and observed its polymerization in the same polymerizing conditions on liposomes, but with a monomer/initiator **3**/**1** ratio of 8750 in tetraborate buffer, since a ratio of 3500 led to a reaction that was too slow. However, in both cases we found that performing ROMP with this monomer gave predominantly polymer nodules with elongated shapes (70% of elongated nodules and 30% of spherical nodules), as shown in Figure 3.

To conclude, we report here for the first time the growth of polymer nodules from liposome surfaces by ROMP in aqueous solution. A change in monomer hydrophilicity leads to a change in polymer nodule shape. This paves the way for further investigations on the dynamics induced by directional polymer growth from a liposome surface.

Acknowledgment. This work was financed by an Action Concertée (DRAB) from the Ministery of Research, France. We thank Ewa Paluch, Julie Plastino, and Jérémy Weber for many fruitful discussions.

Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Blocher, M.; Liu, D.; Walde, P.; Luisi, P. L. *Macromolecules* **1999**, *32*, 7332−7334.
(2) Kitano, H.; Chibashi, M.; Nakamata, S.; Ide, M. *Langmuir* **1999**, *15*, 2709−2713.
(3) Kostarelos, K.; Tadros, T. F.; Luckham, P. F. *Langmuir* **1999**, *15*, 369−376.
(4) Roy, B. C.; Fazal, M. A.; Arruda, A.; Mallik, S.; Campiglia, A. D. *Org. Lett.* **2000**, *20*, 3067−3070.
(5) Jung, M.; Hubert, D. H. W.; Veldhoven, E. V.; Frederik, P.; Van Herk, A. M.; German, A. L. *Langmuir* **2000**, *16*, 3165−3174.
(6) Kocks, C.; Gouin, E.; Tabouret, M.; Berche, P.; Ohayon, H.; Cossart, P. *Cell* **1992**, *68*, 521−531.
(7) Bernheim-Groswasser, A.; Wiesner, S.; Golsteyn, R.; Carlier, M.-F.; Sykes C. *Nature* **2002**, *417*, 308−311.
(8) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18−29.
(9) Mingotaud, A. F.; Reculusa, S.; Mingotaud, C.; Keller, P.; Sykes, C.; Duguet, E.; Ravaine, S. *J. Mater. Chem.* **2003**, *13*, 1920−1925.
(10) France, M. B.; Alty, L. T.; Markley, E. T. *J. Chem. Educ.* **1999**, *76*, 659−660.

JA045482J